3-1-2012

Cell membrane and cytoplasmic epidermal growth factor receptor expression in pancreatic ductal adenocarcinoma.

Amit Mahipal
Thomas Jefferson University, amit.mahipal@jefferson.edu

Mary J Mcdonald
Thomas Jefferson University

Agnieszka Witkiewicz
Thomas Jefferson University

Brian I Carr
Thomas Jefferson University, Brian.Carr@jefferson.edu

As submitted to:

Medical Oncology

And later published as:

"Cell membrane and Cytoplasmic Epidermal Growth Factor Receptor Expression in Pancreatic Ductal Adenocarcinoma"

Volume 29, Issue 1, March 29, 2012, pp. 134-139.

PMID: 21264542

Amit Mahipal[1], Mary J. Mcdonald[2], Agnieszka Witkiewicz[2] and Brian Carr[1]

[1]Department of Medical Oncology, Thomas Jefferson University, Philadelphia, PA

[2]Department of Pathology, Thomas Jefferson University, Philadelphia, PA

Corresponding author: Brian Carr, MD,FRCP, PhD, Department of Medical Oncology, Thomas Jefferson University, Bluemle Building 519, 233 South 10th St, Philadelphia, PA 19107, USA; Tel 215 503 8842; Fax 215 503 8755; E-mail: brian.carr@jefferson.edu

Key Words: EGF receptor, pancreatic cancer, invasion, recurrence

Running head: EGFR expression in pancreatic cancer

Abstract

Introduction. The significance of over-expression of epidermal growth factor receptor (EGFR) in pancreatic carcinoma is unclear. In this study, we examined the association between EGFR over-expression (membranous and cytoplasmic), the associated histopathologic features and clinical outcomes in post resection pancreatic cancer patients.

Methods. EGFR expression was determined immunohistochemically in 90 patients who underwent resection for pancreatic cancer. Cytoplasmic expression was considered positive if EGFR expression was seen in the cytoplasm in ≥10% of cells. Cell membrane staining was scored from 0 to 3+, with 2+ and 3+ being considered as membrane over-expression. Overall survival and progression free survival were calculated using the Kaplan-Meier method and survival curves were compared by the log-rank test.

Results. Out of 90 patients, 51 (57%) and 74 (68%) patients had membrane and cytoplasmic EGFR over-expression respectively. There was a statistically significant correlation between cell membrane EGFR over-expression and lymph node positivity

(p=0.03). Patients with membrane EGFR over-expression had a shorter median progression free survival (10.7 vs. 17.0 months, p=0.02) and overall survival (15.9 months v 25.3 months, p=0.17). Cytoplasmic EFGR over-expression was not significantly associated with recurrence or survival.

Conclusions. Membrane EGFR over-expression in resected pancreatic cancer patients was associated with worse clinical outcomes than non over-expression.

Introduction

Pancreatic cancer is the fourth leading cause of cancer death in United States with 36,800 deaths and 43,140 incident cases estimated in 2010.{{193 Jemal,A. 2010}} Despite therapy, the 5-year relative survival rate is approximately 6%. Due to relative lack of early disease-specific symptoms, only a minority of the patients present with surgically resectable disease. Even in patients who undergo pancreatectomy, the majority of the patients develop recurrence with the 5-year overall survival of only 22.5%.{{193 Jemal,A. 2010}} Various genetic and molecular alterations are being investigated to understand the basis of the disease aggressiveness.

Epidermal growth factor receptor (EGFR) is a 170 kDa protein belonging to ErbB family of transmembrane tyrosine kinase growth factor receptors.{{199 Lemoine,N.R. 1992}} It is overexpressed in wide variety of solid tumors including breast, colon, lung and prostate cancer.{{198 Nicholson,R.I. 2001}} Activation of EGFR in tumors results in increased cell proliferation, reduced apoptosis, increased angiogenesis, increased

motility, invasion and metastatsis.{{200 Kopp,R. 2003}} The EGFR overexpression is observed in 30% to 90% of pancreatic cancers, assayed by immunohistochemistry techniques.{{201 Faller,B.A. 2009}} The cell membrane EGFR overexpression has been demonstrated to be associated with various clinicopathological features. {{187 Yamanaka,Y. 1992; 188 Yamanaka,Y. 1993; 190 Dong,M. 1998; 183 Uegaki,K. 1997; 189 Gansauge,F. 1998; 196 Kuniyasu,H. 2001; 195 Tobita,K. 2003; 186 Ueda,S. 2004; 192 Bloomston,M. 2006; 194 Takikita,M. 2009; 191 Zhang,L. 2002}} However, the effect of membrane EGFR overexpression on clinical outcomes is not well defined. The association between cytoplasmic expression of EGFR and clinicopathologic features in patients with pancreatic cancer has been reported in only one prior study.{{186 Ueda,S. 2004}}

In the current study, we investigated the association between cell membrane and cytoplasmic EGFR overexpression and pathologic features in patients with primary pancreatic cancer undergoing surgical resection. We also examined the role of EGFR overexpression in the prognosis of patients with resected pancreatic cancer.

Methods

Patients

Ninety patients who underwent surgical resection for pancreatic cancer at Thomas Jefferson University from April 2008 to April 2010 were included in the study. All the cases were histologically diagnosed as ductal adenocarcinoma. The patients who did not have any event (recurrence or death) were censored at the last date of follow-up. For pathologic and immunohistochemical evaluation, 10% neutral-buffered formalin fixed

and paraffin-embedded tissue blocks from surgically resected specimens were processed and 5-μm tissue sections were obtained. The tissue sections were stained using routine hemotoxylin and eosin for pathologic diagnosis. The study was approved by Institutional review Board at Thomas Jefferson University.

Immunohistochemistry

Immunohistochemical staining for EGFR was performed on a total of 90 cases using the EGFR PharmDx^{TM} kit (Dako). Specimens were evaluated for both cytoplasmic and membranous immunostaining by a pathologist blinded to the results at Thomas Jefferson University Hospital. Cytoplasmic overexpression was considered positive if EGFR expression was noted in the cytoplasm in ≥10% of tumor cells. Cell membrane EGFR staining was divided into four categories based on intensity and completeness of staining as follows: 0 (no membrane staining or membrane staining in less than 10% of tumor cells), 1+ (incomplete membrane staining in ≥ 10% of cells), 2+ (complete, weak or moderate membrane staining in ≥ 10% of cells) and 3+ (complete, strong staining in ≥ 10% of cells) (Figure 1). Scores of 2+ and 3+ were considered membranous EGFR overexpression.

Statistical Analysis

Descriptive statistics were initially used to characterize the cohort. Chi-square test was used to determine the association between EGFR overexpression and pathologic features. Proportional hazard regression analyses were used to estimate hazard ratios (HR) and 95% confidence intervals (95% CI). Progression-free survival (PFS) and overall survival

(OS) was evaluated using Kaplan-Meier survival curves and differences in survival were tested using log-rank tests. The two sided p-value of 0.05 was used for statistical significance. All statistical analysis was done using SAS statistical software (SAS Institute, Inc., Cary, NC).

Results

Table 1 describes the demographic and pathologic features of the 90 patients of this study. The median age of the study cohort was 68 years (Range: 37-92 years). Thirty seven (41.1%) patients were female and 53 (58.9%) patients were male. The number of patients with AJCC stage I, II, III and IV were 8 (9.2%), 79 (87.8%), 1 (1.1%) and 2 (2.2%) respectively. Seventy seven patients underwent pylorus-preserving pancreaticoduodenectomy and 13 patients underwent distal pancreatectomy. Cell membrane EGFR overexpression was observed in 51 (56.7%) patients. Sixty four (71.1%) patients had cytoplasmic EGFR overexpression. The relationship between EGFR expression and pathologic features is summarized in Table 2. The association between cell membrane EGFR overexpression and lymph node positivity was statistically significant ($p=0.03$). No significant association of cell membrane EGFR overexpression was found with tumor grade, local invasion, margin positivity, vascular invasion, perineural invasion or stage. For cytoplasmic EGFR overexpression, statistically significant association was observed with positive margin only ($p<0.01$).

The median PFS and OS for the study cohort were 11.8 months and 17.1 months respectively. The median follow up time was 15.5 months. Fifty two (58%) patients had either recurrence or death during the study follow up. Histological grade, lymphovascular

invasion and stage were significantly associated with survival. Figure 2 and 3 demonstrates the Kaplan-Meier PFS and OS curves stratified by membrane EGFR overexpression respectively. Membrane EGFR overexpression was associated with statistically significant shorter PFS (median PFS: 10.7 vs. 17.0 months, p=0.02). The median OS for patients with cell membrane EGFR overexpression was also much shorter than the OS in patients without EGFR overexpression (median OS: 15.9 months v 25.3 months, p=0.08), but this difference was not statistically significant, likely due to the small patient numbers (Table 3). For recurrence and death, the HR for patients with cell membrane EGFR overexpression (as compared to no EGFR overexpression) was 1.97 (95% CI: 1.09-3.55) and 1.82 (0.92-3.62) respectively. Cytoplasmic EGFR overexpression did not correlate with PFS (median PFS: 11.7 months v 14.4 months, p=0.30). The corresponding HR was 1.39 (0.74-2.61) without significant difference in survival between patients with or without cytoplasmic EGFR staining (p=0.72). The median OS was 17.1 months in patients with cytoplasmic EGFR overepression and 22.6 months in patients without EGFR overexpression (HR:1.13; 95% CI:0.57-2.27).

Discussion

Few retrospective studies have reported the relationship between EGFR overexpression and clinical outcomes in patients with pancreatic cancer.{{187 Yamanaka,Y. 1992; 188 Yamanaka,Y. 1993; 190 Dong,M. 1998; 183 Uegaki,K. 1997; 189 Gansauge,F. 1998; 196 Kuniyasu,H. 2001; 195 Tobita,K. 2003; 186 Ueda,S. 2004; 192 Bloomston,M. 2006; 194 Takikita,M. 2009}} Membrane EGFR overexpression has been demonstrated to be

associated with higher stage and more aggressive tumors.{{195 Tobita,K. 2003; 191 Zhang,L. 2002}} However, the effect of EGFR overexpression on recurrence and survival in patients with pancreatic cancer remains unclear. Only three out of ten studies reported significantly worse survival in patients with membrane EFGR overexpression than without it (Table 4).{{187 Yamanaka,Y. 1992; 188 Yamanaka,Y. 1993; 190 Dong,M. 1998}} In the current study, the resected pancreatic cancer patients with membrane EGFR overexpression had worse clinical outcomes. The PFS was significantly shorter and there was trend towards poorer survival with membrane EFGR overexpression. There was significant association between lymph node involvement and membrane EGFR overexpression.

To our knowledge, this is only the second study to evaluate the cytoplasmic EGFR overexpression in resected pancreatic cancer patients. In the previous study, patients with cytoplasmic EGFR overexpression had shorter overall survival.{{186 Ueda,S. 2004}} However, in our study, we did not find any statistically significant difference in recurrence or survival in relation to cytoplasmic EGFR overexpression. The cytoplasmic staining of EGFR has been demonstrated to be associated with a poor prognosis in patients with thyroid carcinoma and lung carcinoma. {{202 Piyathilake,C.J. 2002; 203 Akslen,L.A. 1993}} EGFR is internalized after the interaction with the ligand and completion of growth stimulatory signals and is subsequently degraded in lysosmal compartment. Thus, cellular localization pattern of EGFR may be of clinicopathologic significance and its role as a prognostic and predictive marker needs to be further evaluated.

Membrane EGFR expression is associated with poorer prognosis in head and neck, ovarian, cervical, bladder, oesophageal, breast, colorectal and gastric cancers.{{198 Nicholson,R.I. 2001}} EGFR plays an important role in the growth of various human cancers including pancreatic cancer.{{183 Uegaki,K. 1997}} The co-expression of EGFR and its ligand may function as an autocrine loop to constantly stimulate cell proliferation and blockade of EGFR activity has been shown to decrease the growth and metastases of human pancreatic tumor xenografts and enhance the antitumor activity of gemcitabine.{{199 Lemoine,N.R. 1992; 204 Bruns,C.J. 2000; 205 Ng,S.S. 2002}} Erlotinib, a small molecule EGFR-specific tyrosine kinase inhibitor, has been demonstrated to provide modest survival benefit in patients with locally advanced or metastatic pancreatic cancer.{{185 Moore,M.J. 2007}} However, cetuximab, a monoclonal EGFR antibody did not improve survival in patients with advanced pancreatic cancer.{{197 Xiong,H.Q. 2004}} In neither of these studies, did membrane EGFR expression predict tumor response to targeted therapy. Cytoplasmic EGFR expression was not reported. This suggests that in advanced stage pancreatic cancer patients, membrane EGFR expression is not a predictive marker for response to erlotinib or cetuximab. The role of both membrane and cytoplasmic EGFR expression in the adjuvant setting still needs to be examined.

Despite some recent advances in medical treatment, the prognosis of patients with pancreatic cancer remains dismal. Surgery has curative potential but most of the patients present with an advanced, unresectable stage. It is important to develop molecular

markers that can predict clinical outcomes. This may help to direct more aggressive approaches in high risk patients. We think that future clinical trials involving EGFR inhibitors should incorporate systematic evaluation of both cytoplasmic and membrane EGFR expression.

Table 1. Clinicopathologic features of the study population

Parameter	Number (%)
Age >65 years	51 (56.7)
Gender	
Male	53 (58.9)
Female	37 (41.1)
Race	
White	82 (91.1)
Other	8 (8.9)
Site	
Head	73 (81.1)
Body or/and tail	17 (18.9)
Grade	
1	5 (5.6)
2	53 (58.9)
3	32 (35.6)
Stage	
Ia	4 (4.6)
Ib	4 (4.6)
IIa	16 (17.8)
IIb	63 (70.0)
III	1 (1.1)
IV	2 (2.2)
Lymph node involvement	64 (71.1)
Pancreatitis	59 (65.6)
Cell membrane EGFR overexpression	51 (56.7)
Cytoplasmic EGFR overexpression	64 (71.1)

Table 2. Association between cell membrane and cytoplasmic EGFR overexpression and pathologic features.

Parameter	Membrane overexpression			Cytoplasmic overexpression		
	Present	Absent	p	Present	Absent	p
Local invasion						
yes	43	31	0.55	52	22	0.70
no	8	8		12	4	
Grade 3						
yes	19	13	0.70	24	8	0.54
no	32	26		40	18	
Margin positive						
yes	17	6	0.05	22	1	<0.01
no	34	33		42	25	
Vascular invasion						
yes	27	18	0.52	30	15	0.35
no	24	21		34	11	
Perineural invasion						
yes	42	36	0.17	57	21	0.29
no	9	3		7	5	
Lymph node						
yes	41	23	0.03	42	22	0.07
no	10	16		22	4	
Stage IIb or higher						
yes	41	25	0.08	44	22	0.12
no	10	14		20	4	

$p<0.05$ is considered statistically significant.

Table 3. Association between EFGR expression and clinical outcomes

	Progression Free Survival				Overall survival			
	Median (months)	HR	95% CI	p	Median (months)	HR	95% CI	p
Membrane EGFR overexpression								
Present	10.7	1.97	1.09-3.55	0.02	15.9	1.82	0.92-3.62	0.08
Absent	17.0				25.3			
Cytoplasmic EGFR Overexpression								
Present	11.7	1.39	0.74-2.61	0.30	17.1	1.13	0.57-2.27	0.72
Absent	14.4				22.6			

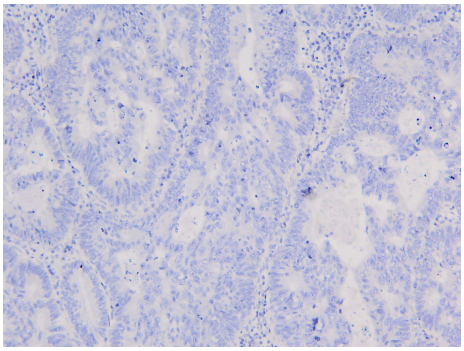
$p<0.05$ is considered statistically significant.

Table 4. Membrane EGFR overexpression and survival in patients with pancreatic cancer.

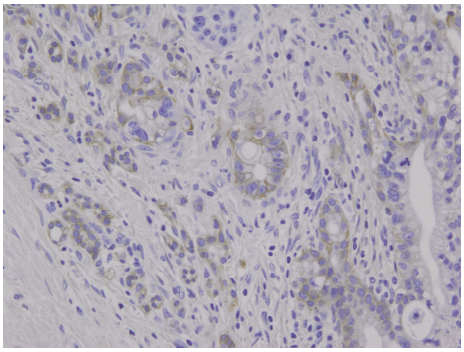
Study	Number of patients	EGFR overexpression, (%)	Prognosis
Yamanka et al{{187 Yamanaka,Y. 1992; }}	25	36	Decreased survival
Yamanka et al{{188 Yamanaka,Y. 1993; }}	87	43	Decreased survival
Dong et al{{190 Dong,M. 1998; }}	57	68	Decreased survival
Uegaki et al{{183 Uegaki,K. 1997; }}	86	44	Trend towards decreased survival
Gansauge et al{{189 Gansauge,F. 1998; }}	82	54	No difference
Kuniyasi et al{{196 Kuniyasu,H. 2001; }}	22	100	No difference
Tobita et al{{195 Tobita,K. 2003; }}	77	42	No difference
Ueda et al{{186 Ueda,S. 2004; }}	76	62	No difference

Bloomston et al{{192 Bloomston,M. 2006; }}	71	69	No difference
Takikita et al{{194 Takikita,M. 2009; }}	154	26	No difference
Current study	90	57	Trend towards decreased survival

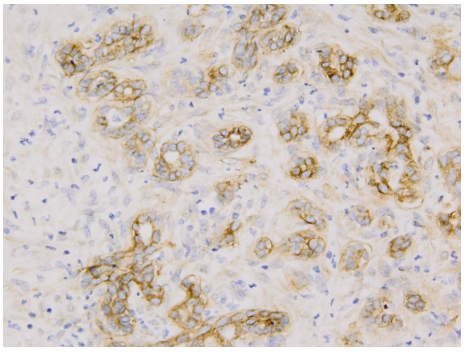
Figure 1. Representative cases of pancreatic cancer demonstrating scores of membrane EFGR expression. A, Score 0; B, Score 1: C, Score 2; D, Score 3.



B



C



D

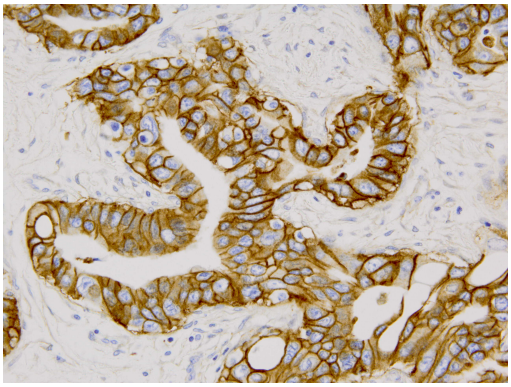


Figure 2. Progression free survival of pancreatic cancer patients stratified by membrane EGFR overexpression

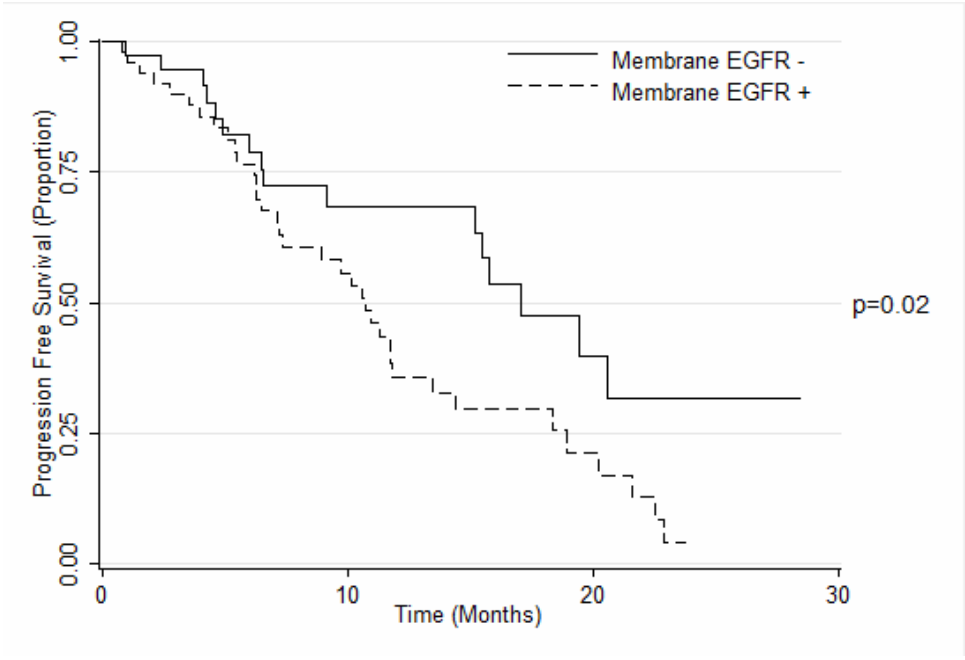


Figure 3. Overall survival of pancreatic cancer patients stratified by membrane EGFR overexpression